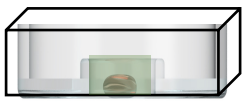
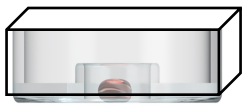
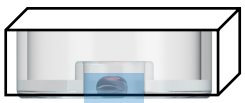
CELLORAMA™

Immunofluorescence Labeling Protocol for 2D Cell Culture

STEP			TEMPERATURE	TIME*
1		Warm-up Fixative (4% Paraformaldehyde), PBS**-Cello™-IF3-Primary Antibody Solution-Secondary Antibody Solution ***-Mounting Medium****	37 °C	10 min
2		Aspirate medium, Fix the sample	37 °C	10 min
3		Incubate with Cello™-IF2	37 °C	3X, 5 min
4		Incubate with Primary Antibody in Cello™-IF2	37 °C	30 min-1 hr
5		Incubate with Cello™-IF	37 °C	3X, 5 min
6		Incubate with Secondary Antibody in Cello™-IF3, Keep in dark	37 °C	30 min-1 hr
7		Incubate with PBS , Keep in dark	37 °C	3X, 5 min
8		Cover with Mounting Medium, Keep in dark	RT	
		Close the lid tightly, Keep in dark until examination.	4 °C	

TOTAL : 2.5-4 hr

*Timings can be adjusted according to the sample and the antibodies.
**PBS: Phosphate Buffered Saline
***Dilute primary and secondary antibodies in CellO-IF.2
***Mounting Medium:1,2 uL Hoechst or DAPI+675 uL PBS + 675 uL Glycerol

TIPS:
-Using a cell culture container with high optical quality is highly recommended (e.g., glass-bottomed dishes and well-plates, multi-chambered slides, CellO ™ - M). The container here represents CellO ™ - M.
-Low-speed orbital shaker can be used to facilitate penetration.
-Most samples are examined on the same day,. Nuclear staining improves after 24 hours in some thick samples.
-Start with the validated dilution rates for each antibody. Adjustment of the dilution rates may be needed based on the nature of the sample and the antibody.
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